INDUCED 1AS.1DL AND 1BS.1DL ROBERTSONIAN TRANSLOCATIONS IN DURUM WHEAT TO IMPROVE ITS END-USE QUALITY

SYNOPSIS

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By

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1. Title: INDUCED 1AS.1DL AND 1BS.1DL ROBERTSONIAN TRANSLOCATIONS IN DURUM WHEAT TO IMPROVE ITS END-USE QUALITY

2. Introduction

Wheat is the second most-consumed cereal crop in the world. Among the three major cereals, it is approximately consumed by 2/3rd of the world population. It is widely grown in India, China, Australia, Canada, etc. Bread wheat provides nutrition and one-fifth of the daily calorie intake. *T. aestivum, T. durum,* and *T. dicoccum* are the three main commercially cultivated wheat species consumed by humans and covering 94.0, 5.0, and 1.0% area respectively. Among the three cultivated wheat in India, hexaploid wheat (*T. aestivum*) is at first place followed by durum wheat and emmer wheat (*T. dicoccum*) based on area and production.

Triticum is a family of Poaceae with species ranging from diploid (2n=2x=14) to hexaploid (2n=6x=42) with basic chromosome number 7. *T. aestivum* L. is hexaploid wheat with genome AABBDD and chromosome 2n=6x=42. The A genome comes from *T. urartu*, the B genome from *Ae. speltoides* or other closely related species (Kerby and Kuspira 1987; Tsunewaki and Ogihara 1983; Zhang et al 2001a), and the D genome from *Ae. tausachii*. Hexaploid wheat evolved from three diploid ancestors via interspecific hybridization and chromosome doubling. *Triticum urartu* (2n=2x=14, AA), a wild diploid wheat species, crossed with another diploid closest unknown wild grass species (2n=2x=14, BB), resulting in the Tetraploid emmer wheat, *Triticum dicoccum* (2n=4x=28, AABB) (Feldman 2001). After several thousand years of emmer wheat domestication, durum wheat (2n=4x=28, AABB) evolved. About 8000 years ago, tetraploid durum wheat (2n=28, AABB) crossed with diploid wild goatgrass (DD), resulting in hexaploid wheat. *T. aestivum* is used to make bread and chapattis.

Durum wheat is a tetraploid (2n=4x=28) (AABB). The D genome confers high gluten strength and bread-making quality to bread wheat. Around 17 million hectares of durum wheat (*Triticum turgidum*) are cultivated annually, yielding around 40 million tonnes of durum wheat. Production of durum wheat is concentrated in the Middle East, North Africa, Asia, and Europe. Because durum wheat is only 8-10% of total wheat production worldwide, it is a minor crop compared to common wheat. It is

used to prepare pasta, macroni, spaghetti, vermicelli, noodle products, halva, upma, idli, dosa. Hexaploid wheat is used to make chapattis, cakes, biscuits, and bread. Most wheat's endosperm or breaks down into fine powdery flour when milled, but durum endosperm holds together. For making pasta, durum is chosen for hardness.

Durum kernels are amber-colored and larger. It is used to make many products all over the world. It is a common food ingredient. Wheat homeologous group 1 chromosome encodes seed storage protein, high molecular weight glutenin, low molecular weight glutenin, and gliadin storage protein subunits (Damania and Payne 1984). These subunits influence processing and nutrition. Interspecific hybridization has been used to improve the protein content and quality of durum wheat. Durum breeding aims to produce varieties with high protein content and improved gluten strength with HMW-GS for pasta and bread making quality. Its gluten strength makes it non-elastic. Gluten is a family of wheat proteins that includes glutenin and gliadin. In wheat, prolamine is called gliadin and glutelin is called glutenin.

Each high molecular weight glutenin subunit (HMWG) is made up of two monomeric gliadins and one polymeric glutenin subunit (LMWG) and high molecular weight glutenin subunit (HMWGs). HMWGs are significant in bread making, according to SDS-PAGE. Long arm of group one homeologous chromosomes contains HMW-GS alleles (1A, 1B, 1D). Ax, Ay, 1Bx, 1By, and 1Dx, 1Dy subunits are found in each locus. Better dough strength and bread-making quality are connected with HMW-GS 1Dx5+1Dy10 pair (Vasil and Anderson 1997).

Intriguingly, *Triticum durum* lacks the D genome, and the *Glu-A1* region has been silenced by transposable element insertion and stop codon within locus (Shewry et al 2006). Gluten must have extensibility and elasticity to expand and retain carbon dioxide formed during baking (Ferrante et al 2006). Pasta requires hard, tenacious, non-elastic gluten. Also, pasta dough absorbs water at a rate of 31-35 percent versus 60% for bread dough.

For bread-making quality, the dough can be firm but also needs elasticity to avoid a doughy product. Durum with weak gluten exhibits more viscous, less elastic dough, low sedimentation value, and low loaf volume (Boggini and Pogna 1989). From the nutritional point of view, durum wheat has a high protein and carbohydrate content as compared to hexaploid wheat. It contains 12-15% of protein and 74-77% of carbohydrates. On the other hand, hexaploid wheat contains 8-12% protein and 70% of carbohydrates. High protein content greater than 13% is generally desirable for high-quality pasta.

The long arm of chromosome 1D carries the *Glu-D1* allele for HMW-GS for 1Dx5+1Dy10, 1Dxt2+1Dx2T, and 1Dx2+1Dy12 which are considered the most desirable alleles for bread-making quality (Payne et al; 1984). Short arms of chromosome 1A and 1B carry *Glu-A3* and *Glu-B3* loci for LMW-GS which are tightly linked to the complex *Gli-1* loci, encoding γ- and ω-gliadins which have a stronger affinity for bread-making quality. Whereas the short arm of chromosome 1D carries the *Glu-D3*

locus with a minor role in determining the end-use quality of wheat (Kamal et al 2009; Payne et al 1984; Pogna et al 2002; Singh and Shepherd 1988).

Robertsonian translocations develop by centric mid-division followed by the fusion of the broken arms from different chromosomes. Several attempts have been made to induce Robertsonian translocations in wheat-rye (1RS.1BL), and wheat-durum (1AS.1DL), Wheat-*Elymus*, Wheat-*Thinopyrum villosum* to induce better agronomic traits. The 1RS.1BL translocation is the source of several disease resistance genes (leaf, stem, and yellow rust, and powdery mildew resistance loci) on the 1RS arm (Lukaszewski 1997). One important reason to induce, 1AS.1DL and 1BS.1DL Robertsonian translocation in durum wheat is to improve processing quality with the addition of glutenin loci on the 1DL arm of wheat.

For developing Robertsonian translocations addition of alien chromosomes is generally required by substitution of its chromosome. A complete set of Langdon durum disomic substitution lines has been developed in Chinese Spring background with substitution of chromosome 1D for 1A and substitution of chromosome 1D for 1B (Joppa et al 1983; Joppa et al 1998). 1AS.1DL Robertsonian translocation was developed by crossing with a lo (scs) a durum cultivar with 1A (1D) substitution line, the F_1 hybrid had been double monosomic chromosome for 1A and 1D. The unpaired chromosomes were undergoing centric breakage fusion to give 1AS.1DL translocation (Maan et al 1999). Monosomic 1D addition lines were developed in durum background PBW114 with 29 (28+1D') chromosomes. Selfing for several generations leads to the development of disomic substitution line 1D for 1A (26+1D''-1A'') and 1D for 1B (26+1D''-1B'') (Dhaliwal et al 1990; Garg et al 2007).

The present study aims to induce the 1AS.1DL and 1BS.1DL Robertsonian translocation in durum wheat through the PBW114-1A+1D and PBW114-1B+1D substitution lines, and the work with the following broad objectives

Objectives:

a. Induce Robertsonian 1AS.1DL and 1BS.1DL translocations in crosses of PBW114-1A+1D and PBW114-1B+1D substitution line with elite durum cultivar.

b. Select 1AS.1DL and 1BS.1DL Robertsonian translocations using morphological, Group-1 homeologous molecular markers and SDS-PAGE analysis.

c. Evaluation of nutritional and end-use quality of durum derivatives with 1AS.1DL and 1BS.1DL translocations.

3. Expected New Knowledge

This study will improve the processing, nutritional quality of the durum wheat through 1AS.1DL and 1BS.1DL Robertsonian translocations. The silencing of *GluA1* and the absence of the D genome makes durum wheat unsuitable for bread-making quality. The objectives of this study are to produce dual-purpose durum wheat lines with high nutritional value as well as bread-making quality. The long arm of 1D Chromosomes carries the glutenin loci *GluD1* which is useful for bread-making quality. Substitution lines of PBW114-1A+1D and PBW114-1B+1D with the long arm of 1D chromosome carry 1Dxt2+1Dy2T subunit which is beneficial for the end-use quality. Short arms of chromosome 1A and 1B carry *Glu-A3* and *Glu-B3* loci for LMW-GS are tightly linked to the complex *Gli-1* loci, encoding γ- and ω-gliadins with stronger affinity for bread-making quality. Our objective is to exploit 1AS, 1BS, and 1DL chromosome arms by inducing 1AS.1DL and 1BS.1DL Robertsonian translocations to improve end-use quality of durum wheat.

4. Review of Literature

Low sedimentation value and low loaf volume are characteristics of durum wheat with weak gluten. The *Glu-A1* locus is silenced, and the chromosome 1D encoded protein necessary for dough elasticity and extensibility is absent (Ceoloni et al 2003). The baking potential of durum wheat has been improved by incorporating 1D protein genes (Lafiandra et al 2000). The HMW-GS subunits are encoded on the long arm of group 1 homeologous chromosomes (1A, 1B, 1D) as *GluA1, GluB1*, and *GluD1* alleles, respectively. To make stronger dough and better bread, the most favorable allele for bread making quality on the long arm of 1D chromosome's has 1Dx5+1Dy10 HMW-GS, followed by t2+2T HMW-GS and 1Dx2+1Dy12 HMW-GS pairs (Vasil and Anderson 1997). Durum wheat has four HMW-GS genes, but due to gene silencing, most genotypes have one to three subunits (Oak and Dexter 2006).

Glu-B1 encodes 7+8, 7+9, 17+18, and 6+8 HMW-GS in most durum wheat cultivars. Combining the Glu-B1 loci (7+8) and (17+18) with the Glu-D1 subunit (5+10), (2+12), and (2t+t2) may improve end-use quality. *Gli-1* encoded gliadins are present on the short arm of group 1 homeologous of wheat (Dhaliwal et al 2002). Compared to bread wheat, durum wheat has a lower percentage of HMW-GS and a higher percentage of LMW-GS. To increase dough strength in durum wheat, increase HMW-GS loci number. The silent gene at *Glu-A1* was replaced with an allelic form expressing both x and y type (5+10, 2+12, other subunits 2t and t2) by swapping 1 D for 1A chromosome (Shewry et al. 2006). The addition of 1D to 1A increased the amount of glutenin and thus the dough strength. Translocation lines with HMW-GS 5+10, 2+12, t2, and 2t have been introduced to replace the null alleles present in the *Glu-A1* locus using chromosome engineering (Ceoloni et al. 2003).

These proteins influence gluten strength, spaghetti cooking, and bread-making quality in durum wheat (Boggini and Pogna 1989; Ito et al 2012). Those with HMW-GS 7+8 and LMW-GS type-2 (45) had larger loaf volume than those with LMW-GS type-1 (42); (Ayala et al 2016; Pea 2002). This means that selecting for improved bread-making quality in durum wheat with *Gli-B3* loci can use *Glu-B1* encoded HMW-GS alleles. Glutenin/gliadin ratio increases with D genome proteins (HMW-GS 5+10 or HMW 2+12) in durum (Lafiandra et al. 2000). Adding glutenin enriched fraction to base flour increased the ratio of polymeric to monomeric gliadin (glutenin to gliadin) (Uthayakumaran et al 1999).

The 1AS.1AL-1DL translocation line of durum wheat has *Glu-D1* (1Dx5+1Dy10) subunit overexpression, which is detrimental to bread-making quality because the translocation lines loaf volume is identical to the parents. Sears created a monosomic all chromosomes substitution stock in Chinese Spring wheat (Klindworth et al 2005). A complete set of disomic substitution lines for chromosome 1D for 1A and chromosome 1D for 1B was prepared in durum background, crossing Langdon a durum cultivar by the monosomic line of Chinese Spring and have improved dough strength by introducing 1Dx2+1Dy12 subunit (Joppa and Williams 1988). On the other hand *Ae. tauschii* accession TA1588, TA1591, and TA3754 have *Glu-D1* alleles, encoding 1t+12t, 1t+10t, and 2t+T2 subunits which are beneficial for dough strength (Xu et al; 2010).

4.1 Introduction about the Material

A monosomic 1D addition line of durum cultivar PBW114 with 29 chromosomes carried genes for red glume color located on the short arm of chromosome 1D (Dhaliwal et al. 1990). This line self-pollinated for several generations and cytological analysis revealed that three out of four advanced generation lines 1D3596, 1D3597, and 1D3601 had to substitute chromosome 1D for 1A and other lines 1D3598 had a substitution of chromosome 1D for 1B (Table 1) (Garg et al. 2007). SDS-PAGE analysis of parent durum PBW114 indicates that the parent durum PBW114 carries 6+8 HMW-GS encoded by the Glu-B1 locus and the line. Line 1D3596, 1D3597, and 1D3601 carry two extra subunits of HMW-GS in addition to 6+8 subunits encoded by the 1D chromosome carrying 2t+2T subunit and line 1D3598 have a substitution of *Glu-B1* (6+8) encoding proteins with *Glu-D1* (2t+T2) subunit.

4.2 Robertsonian Translocation

Robertsonian translocations are created by combining broken arms from distinct chromosomes (Kopecky and Lukaszewski 2019). For wheat-alien Robertsonian translocation, the appropriate wheat and alien chromosomes are monosomic (unpaired). Unpaired chromosomes are univalent in remote hybridization. Unpaired monosomy chromosomes are prone to focal breakage in anaphase II. The univalent undergoes chromosomal or chromatid segregation at anaphase-I (Fig. 1) and is misdivided in 6-

7 percent of the pollen mother cell at anaphase-II. The Robertsonian translocation in wheat-rye showed that centric breakage fusion can occur anywhere within the major constrictions without impacting centromere function (Zhang et al 2001b).

Table 1 *Triticum durum* cv. PBW114 with 1D substitution line

Sr. No	Name of the substitution line	Chromosome constitution	Substitution of chromosome	Name of substitution line
1.	1D3596	30 chromosome (12A + 14B + Two 1D + 2′ mini-chromosomes)	1D chromosome substituted for 1A	Disomic substitution line
2.	1D3597	28 chromosome (12A + 14B + Two 1D)	1D chromosome substituted for 1A	Disomic substitution line
3.	1D3601	28 chromosome (12A + 14B + Two 1D)	1D chromosome substituted for 1A	Disomic substitution line
4.	1D3598	28 chromosome (14A + 12B + Two 1D)	1D chromosome substituted for 1B	Disomic substitution line

Wheat-Elymus Robertsonian translocation occurs in monosomic condition (Zhao et al 2010). Robertsonian translocation occurs in 4-20% of double monosomic situations. Robertsonian translocation is best induced by self-pollination of double monosomic plants. The researcher tried to produce Robertsonian translocations in wheat. Dough strength was improved by translocating 1EL-1AS in *Thinopyrum*-Chinese spring. Wheat end-use quality is improved by HMWGS genes on the Thinopyrum 1EL chromosome. This method has also been used to impart leaf rust and stem rust resistance. A disomic foreign addition line of *Th. elongatum* chromosome 1E was obtained in Chinese Spring wheat. *Pm21* and *Sr52* resistance is found in *Dasypyrum villosum* (L.), a wild relative of wheat. Wheat-*Dasypyrum villosum* Robertsonian translocation T6AS•6V#3L for stem rust resistance with Sr52 gene was introduced into wheat (Liu et al 2011; Qi et al 2011). The *Pm21* gene from *Daspyrum villosum* was introduced to bread wheat by a T6AL•6V#2S Robertsonian translocation (Chen et al 1995). Durum wheat 1RS.1BL translocation was introduced by crossing tetraploid “Cando” with hexaploid “Veery” (Friebe et al 1987). 1AS.1DL translocation line was established in ‘Rhino' triticale and transplanted into durum background to boost durum quality (Blanco et al 2002; Lukaszewski and Curtis 1992; Lukaszewski and Curtis 1994). An elite durum line will be crossed with a PBW-1A+1D disomic substitution line to produce a double monosomic F_1 hybrid with univalent 1A and 1D chromosomes. Table 2 and Table 3 show the chromosomal makeup of the F_1 hybrid F_2 plants. An elite durum line will be crossed with a PBW-1B+1D disomic substitution line to produce a double monosomic F_1 hybrid with 1B and 1D chromosomes univalent. Tables 4 and 5 show the chromosomal composition of the F_2 plants of F_1 hybrids.

5. Technical Programme

5.1 Objective 1: To Induce Robertsonian 1AS.1DLand 1BS.1DL translocations in crosses of PBW114-1A+1D and PBW114-1B+1D substitution line with elite durum cultivars.

Material: PBW114-1A+1D, PBW114-1B+1D substitution line.

Donor Parents: 1D3596, 1D3597, 1D3598, 1D3601.

Recipient Parents: PDW233, PDW291, PDW274, PDW275, PDW283, PDW314**.**

Table 2 Chromosome constitution of F_2 plants of F_1 hybrid of PBW114-1A+1D'' substitution line × elite durum line

		Male Transmission (♂)			
		1A		**1D**	
		48% 1A (14 chromosome)	2% – (13chromosome)	48% 1D (14 chromosome)	2% – (13 chromosome)
Female transmission (♀) **1A**	12.5% 1A (14 chromosome)	6% 1A1A (Disomic)	0.25% 1A – (Monosomic)	6% 1A1D (Disomic)	0.25% 1A– (Monosomic)
	33.6% – (13 chromosome)	18% 1A– (Monosomic)	0.75% — (Nullisomic)	18% 1D – (Monosomic)	0.75% – – (Nullisomic)
1D	12.5% 1D (14 chromosome)	6% 1A1D (Disomic)	0.25% 1D- (Monosomic)	6% 1D1D (Disomic)	0.25% 1D – (Monosomic)
	33.6% – (13 chromosome)	18% 1A– (Monosomic)	0.75% – – (Nullisomic)	18% 1D- (Monosomic)	0.75% – – (Nullisomic)

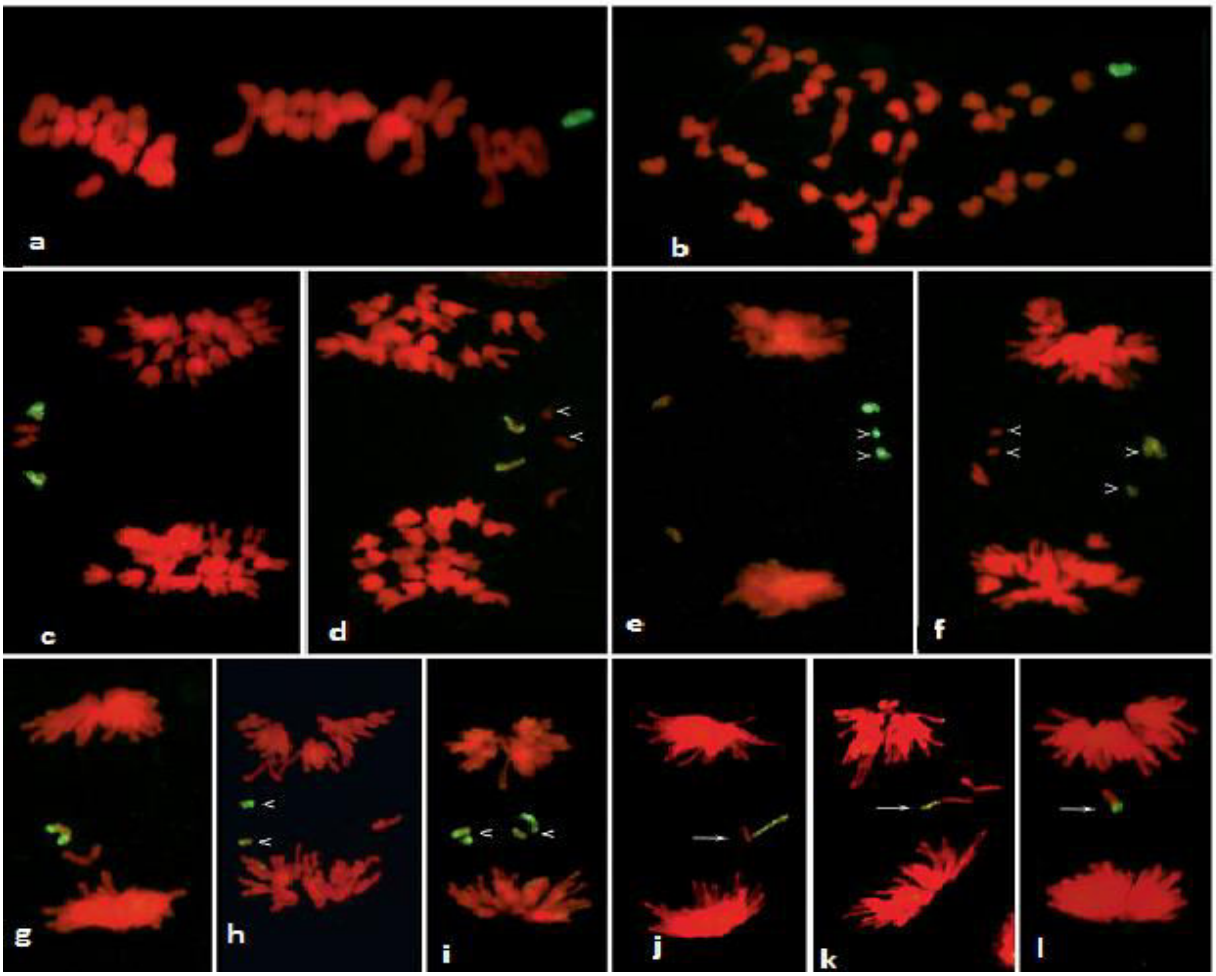

Figure 1: Diagrammatic representation of Robertsonian Translocation (Image taken from Friebe et al; 2005). **a)** metaphase 1: two univalents are present **b-f)** anaphase I: chromatid segregation both univalents goes on opposite pole **g-i)** half tetrad at anaphase II **k)** joining of broken ends **l)** Robertsonian translocation

Table 3 Proportion of F_2 plants with chromosome constitution and glume color

Chromosome constitution	**The proportion of F_2 Plants**	**Glume color**
1A1A (Disomic)	6%	White
1A- (Monosomic)	36.5%	White
1A1D (Disomic)	12%	Red
1D- (Monosomic)	36.5%	Red
1D1D (Disomic)	6%	Red
-- (Nullisomic 1A, Nullisomic 1D, Double nullisomic	1.5%, 0.85% and 0.5%	

5.1.1 Name of experiment 1: Validation of donor Parents with disomic substitution line (PBW114-1A+1D) and (PBW114-1B+1D) by cytological analysis.

5.1.2 Location: Central Laboratory-I, Department of Genetics-Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture

5.1.3 Methodology: The donor parents' chromosomes were counted and paired. The spikes will be fixed in Carnoy's fixative (ethanol: chloroform : acetic acid::6:3:1) for 24 hours before being transferred to 70% ethanol solution. After crushing anthers in a 2 percent acetocarmine solution, pollen mother cells (PMCs) will be scored for chromosome number and pairing behavior using a compound microscope (Magnus, New Delhi, India). Photographs taken with a digital camera (12J875).

Table 4 Chromosome constitution of F_2 plants of F_1 hybrid of PBW114-1B+1D'' substitution line × elite durum line

Female transmission (♀)		Male Transmission (♂) 1B		1D	
		48% 1A (14 chromosome)	2% – (13 chromosome)	48% 1D (14 chromosome)	2% – (13 chromosome)
1B	12.5% 1A (14 chromosome)	6% 1B1B (Disomic)	0.25% 1B – (Monosomic)	6% 1B1D (Disomic)	0.25% 1B – (Monosomic)
	33.5% – (13 chromosome)	18% 1B– (Monosomic)	0.75% – – (Nullisomic)	18% 1D – (Monosomic)	0.75% – – (Nullisomic)
1D	12.5% 1D (14 chromosome)	6% 1B1D (Disomic)	0.25% 1D– (Monosomic)	6% 1D1D (Disomic)	0.25% 1D – (Monosomic)
	33.6% – (13 chromosome)	18% 1B– (Monosomic)	0.75% – – (Nullisomic)	18% 1D- (Monosomic)	0.75% – – (Nullisomic)

Table 5 Proportion of F_2 plants with chromosome constitution and glume color

Chromosome constitution	The proportion of F_2 Plants	Glume color
1B1B (Disomic)	6.0%	White
1B- (Monosomic)	36.5%	White
1B1D (Disomic)	12.0%	Red
1D- (Monosomic)	36.5%	Red
1D1D (Disomic)	6.0%	Red
-- (Nullisomic 1D, Nullisomic 1B, Double Nullisomic	1.5%, 0.85% and 0.5%	

5.1.4 Observations: The derivatives have been analyzed for chromosome number and pairing behavior.

5.1.5 Statistical analysis: Nil

5.1.1 Name of experiment 2. Validation of donor Parents with disomic substitution line (PBW114-1A+1D) and (PBW114-1B+1D) by group-I morphological and molecular marker analysis

5.1.2 Location: Central Laboratory-I, Department of Genetics-Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture

5.1.3 Methodology: The dominant and SSR markers have been used to validate the chromosome 1D substitution lines 1D3596, 1D3597, 1D3601, 1D3598 group 1 homeologous marker of 1A, 1B, and 1D chromosome.

5.1.4 Observations: The have been analyzed by the banding pattern of the PCR result

5.1.5 Statistical analysis: Nil

5.1.1 Name of experiment 3. Analysis of Micro SDS sedimentation test (MST) and high molecular weight glutenin subunit (HMWGS) in donor and recipient parental lines.

5.1.2 Location: Central Laboratory-I, Department of Genetics-Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture

5.1.3 Methodology: The micro SDS sedimentation test (MST) values have been recorded in the donor and recipient parents (Dick et al 1983). The (HMWGS) analysis of parental lines has been analyzed on SDS PAGE gel (Singh et al 1991).

5.1.4 Observation: The micro SDS sedimentation test (MST) values have been recorded in millimeters Table 6 and the (HMWGS) analysis have been recorded by banding pattern.

5.1.5 Statistical analysis: Nil

Table 6 List of Micro SDS sedimentation test (MST) value of donor and recipient parents

Sr. No	Recipient parents	MST (mm)	Donor Parents	MST (mm)
1	PDW233	31 mm	1D3596	64 mm
2	PDW291	29 mm	1D3597	66 mm
3	PDW274	43 mm	1D3601	66 mm
4	PDW275	42 mm	1D3598	47 mm
5	PDW283	35 mm		
6	PDW314	34 mm		

5.1.1 Name of experiment 4. Inducing 1AS.1DL and 1BS.1DL Robertsonian translocation in crosses of PBW114-1A+1D (1D3596, 1D3597, 1D3601, and PBW114 substitution line) and PBW114-1B+1D (1D3598) substitution line with elite durum cultivar.

5.1.2 Location: Maccher Experimental Farm and Central Laboratory-I, Department of Genetics-Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture

5.1.3 Methodology: The F_1 generation has been developed by crossing PBW114-1A+1D (1D3596, 1D3597, 1D3601) and PBW114-1B+1D (1D3598) with elite durum cultivars PDW233, PDW291, PDW274, PDW275, PDW283, PDW314, and the F_1 plants will be analyzed for chromosome number and pairing behavior. The donor parents' chromosomes were counted and paired. The spikes will be fixed in Carnoy's fixative (ethanol: chloroform : acetic acid::6:3:1) for 24 hours before being transferred to 70% ethanol solution. After crushing anthers in a 2 percent acetocarmine solution, pollen mother cells (PMCs) will be scored for chromosome number and pairing behaviour using a compound microscope (Magnus, New Delhi, India). Photographs taken with a digital camera (12J875).

5.1.4 Observation: The F_1 Plants have been analyzed for chromosome number and the pairing behavior. All the plants will be analyzed for double monosomic conditions suitable for Robertsonian translocation.

5.1.5 Statistical analysis: Nil

5.2 Objective 2: To Select 1AS.1DL and 1BS.1DL Robertsonian translocations using morphological and group-1 homeologous molecular markers and SDS-PAGE analysis

5.2.1 Name of experiment: 1 Selection of 1AS.1DL and 1BS.1DL translocation using group I homeologous marker.

5.2.2 Location: Maccher Experimental Farm and Central Laboratory-I, Department of Genetics-Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture.

5.2.3 Methodology: For 1AS.1DL Robertsonian translocation a group of dominant and SSR group 1 homeologous marker that cover all the regions of the short and long arm of chromosome 1A and 1D will be used and 1AS.1DL Robertsonian translocation will be selected. The (HMWGS) analysis of translocation line will be analyzed on SDS-PAGE gel (Singh et al. 1991).

5.2.4 Observations: The Robertsonian translocation will be selected on the banding pattern of PCR results and a map will be prepared with the help of PCR marker result and the (HMWGS) analysis will be recorded by banding pattern.

5.2.5 Statistical analysis: Nil

5.3 Objective: 3 Evaluation of end-use quality and nutritional value of durum derivative with 1AS.1DL and 1BS.1DL translocation

5.3.1 Name of experiment 1: Micro SDS sedimentation test (MST) and high molecular weight glutenin subunit (HMWGS) analysis of Robertsonian translocation line.

5.3.2 Location: Central Laboratory-I, Department of Genetics-Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture

5.3.3 Methodology: The micro SDS sedimentation test (MST) values will be recorded in 1AS.DL and 1BS.1DL translocation line with comparison to the HD2967 (Dick et al. 1983). The nutritional quality parameters viz. protein, carbohydrate, iron, and zinc content will be recorded by the biochemical analysis by using a standard plot.

5.3.4 Observation: The micro SDS sedimentation test (MST) values will be recorded in millimeters and the nutritional values will be recorded by the biochemical analysis.

5.3.5 Statistical analysis: Nil

6. Collaboration (if any): Nil

7. References

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Report of the Research Degree Committee on Synopsis of Master's /Ph.D Thesis/Dissertation Research Problem

We have critically examined the synopsis of Thesis/Dissertation Problem entitled :**INDUCED 1AS. 1DL AND 1BS. 1DL ROBERTSONIAN TRANSLOCATIONS IN DURUM WHEAT TO IMPROVE ITS END-USE OF QUALITY** submitted by : **Ms. Pooja Saini** Registration No :**BS18PSBT002** for M.Sc/M. Tech/Ph.D degree in the subject of: **Biotechnology** to the Dr. KS Gill Akal College of Agriculture of the Eternal University, Baru Sahib(HP). It is certified that the research problem in original; programme of work is properly planned, feasible to carry out and is of good academic and practical significance.

Ms/Mr.: **Ms. Pooja Saini** gave a presentation on the above mentioned research problem to the Research Degree Committee and other faculty members and students on **November 25, 2021** and his/her performance is Satisfactory/~~Unsatisfactory~~.

Synopsis is recommended for acceptance in by the Eternal University.

(Signature, Name and Address of of External Expert)

(Dr. Joy. K. Roy)
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1. _____
(Chairperson, Research Degree Committee)

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6. _____
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Recommendation

(Dean, Post-graduate Studies)

Approved/Not Approved

Vice Chancellor